

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

PCT

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION See paragraph 2 below

International application No.
PCT/GR2004/000051

International filing date (day/month/year)
18.10.2004

Priority date (day/month/year)
16.10.2003

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
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1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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10/576354

IP20 Rec'd PCT/PTO 17 APR 2006

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/GR2004/000051

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing:
 - ☒ contained in the international application as filed.
 - ☐ filed together with the international application in computer readable form.
 - ☒ furnished subsequently to this Authority for the purposes of search.
3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/GR2004/000051

Box No. II Priority

1. ☐ The following document has not been furnished:
- ☐ copy of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(a)).
 - ☐ translation of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(b)).
- Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.
2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.
4. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-10
	No: Claims	
Inventive step (IS)	Yes: Claims	9
	No: Claims	1-8,10
Industrial applicability (IA)	Yes: Claims	1-10
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43bis.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43bis.1 and 70.9)

see form 210

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/GR2004/000051

Re Item I

Basis of the report

The sequences SEQ ID Nos: 1 & 2 filed in the sequence listing on paper together with the application differs from the sequences mentioned in claim 1 of the application. The search has been performed for the sequences mentioned in claim 1. The electronically filed sequences are identical to the sequences in the sequence listing.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document/s/:

- D1: RYAN P ET AL: "PCR detection of Mycobacterium paratuberculosis in Crohn's disease granulomas isolated by laser capture microdissection" GUT, vol. 51, no. 5, November 2002 (2002-11), pages 665-670, XP001205548 ISSN: 0017-5749
- D2: BULL TIM J ET AL: "Characterization of IS900 loci in Mycobacterium avium subsp. paratuberculosis and development of multiplex PCR typing" MICROBIOLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB, vol. 146, no. 9, September 2000 (2000-09), pages 2185-2197, XP002173061 ISSN: 1350-0872
- D3: EP-A-1 223 225 (STICHTING NEDERLANDS INSTITUUT VOOR ZUIVELONDERZOEK) 17 July 2002 (2002-07-17)

1. None of the cited documents disclose a primer with the sequence P1N, P2N, P3N or P4N and claims 1-10 are therefore novel.
2. Each of the above documents D1-D3 and a high number of documents that are not cited disclose primers used in PCR and nested PCR which bind within and amplify a portion of the IS900 insertion element of M.avium subsp. paratuberculosis.

The primers P1N, P2N, P3N and P4N do not appear to have any special effect when used alone or in combination with other primers, but show a surprising high sensitivity

and specificity when used in combination in a nested PCR. Thus, the ISA is of the opinion that the problem solved by each of the primers in claim 1 is the provision of an alternative PCR primer binding to IS900.

The design of PCR primers for amplifying a region with a known sequence is considered to belong to the routine tasks which would be obvious to the skilled person wishing to find alternative primers for the amplification of a certain sequence and the ISA therefore finds that each of the primers represent a selection which would be one of the obvious options to the skilled person wishing to provide alternative primers. Thus, claim 1 is not considered inventive.

3. The application does not disclose any surprising specificity or sensitivity of any of the primer combinations of claim 2, apart from the combination employing all 4 primers and claim 2 therefore lacks an inventive step for the same reasons as claim 1.
4. The subject-matter of claims 3-8 and 10 refers to claim 1 and/or 2 and relate to features which are trivial in the field and therefore do not involve an inventive step for the same reasons as claim 1 and/or claim 2.
5. The subject-matter of claim 9 appears to involve an inventive step in view of the surprising sensitivity, reproducibility and specificity (see pages 7-8) compared to prior art PCR tests for detection of MAP. The prior art does not contain any pointer to the use of the specific primers P1N, P2N, P3N and P4N for obtaining a test with improved characteristics and the subject-matter of claim 9 therefore would not be an obvious solution to the skilled person.
6. The subject-matter of claims 1-10 is considered industrially applicable.